IS BIOFILM EFFECTING YOUR PROCESS OR APPLICATION?

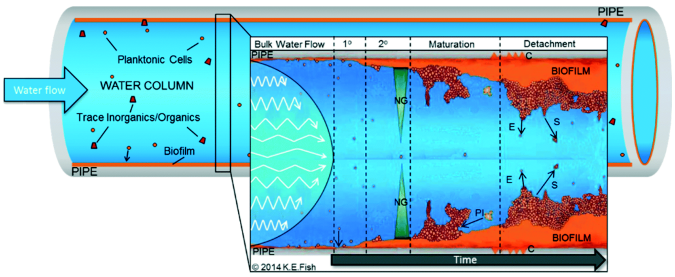
One of the early applications of JC 9450 was treating well water fed farm animals. In doing so, it was discovered that regardless of BOD, COD or TOC, an injection rate to reach a target ORP of +500 mV to +550 mV was more than satisfactory in handling the organic loading, prevent algae growth where the animals were drinking as well as prevent scale build up on drinkers utilized by small animals with the added benefit that the animals were drinking more water.

to

As part of the investigation, the ORP at the terminal end of the distribution system was observed. On a system not having been exposed to JC 9450, the initial ORP was in the range of +100 mV and depending on the length of the distribution system, it took 4-6 weeks for the terminal end ORP to rise to a max +520mV after starting the chemical feed of JC 9450.

Further investigation revealed that the biofilm had been removed from the distribution system. To better understand the effectiveness of JC 9450 against biofilm, this presentation was designed to discuss in detail the nature of biofilm, its susceptibility to chemical disinfectants and why JC 9450 will remove biofilm better than any chemical treatment program available.

Biofilm



Attachment of a bacterium cell to a surface is a signal for the expression polysaccharide specific genes which then anchors and encases the cell in an adhesive polysaccharide polymeric matrix excreted by the cell. This matrix is referred to as “extracellular polymeric substance” (EPS). EPS helps prevent detachment, traps nutrients and protects the growing colony from detrimental chemicals. Upon adhesion, synthesis of exopolysaccharide is coupled to cell division, which leads to the formation of microcolonies. Networks of water channels are formed in biofilms; these channels are responsible for the exchange of nutrients and metabolites with the bulk fluid.

The chemical structure of EPS varies among different types of organisms and is also dependent on environmental conditions, i.e., filters, distribution systems, type of surface, available AOC (assimilable organic carbon), VOC (volatile organic carbon), inorganics, exposure to disinfectants, …. Etc.

EPS is generally heterogeneous, frequently containing more than one distinct microenvironment. EPS with both aerobic as well as anaerobic strata are common and often contain many layers with embedded bacteria of either a single, but mostly of multiple, microbial species that form an interdependent structured community, capable of coordinated and collective behavior. Although microbial interactions among bacteria have been studied primarily in planktonic culture systems, these are more likely to occur in multispecies biofilms in which genetically distinct bacteria may become attached to one another via specific molecules. Bacterial interactions may be accomplished through extracellular compounds whose sole role is to influence gene expression, metabolic cooperativity and competition, physical contact, and the production of antimicrobial exoproducts. One or all of these may be occurring simultaneously and begin to influence a biofilm during the initial stages of formation,

bacterial attachment, and surface colonization and continue to influence the structure and physiology of the biofilm as it develops.

Commensal relationships enable microbes that would normally be susceptible and sensitive to disinfection, to survive. *Mycobacterium chelonae* increased the cultivability of *Legionella pneumophila*. *M. chelonae* and *Sphingomonas sp.* also helps *Helicobacter pylori* to maintain cultivability for at least 24 hours. In contrast, *H. pylori* demonstrated that it loses cultivability in less than 24 hours when in monospecies or in dual-species biofilms with *V. paradoxus*, *Acidovorax sp.* and *Brevundimonas sp.*, It appears that *M. chelonae* may have an important role in the survival of both pathogens in drinking water. The presence of some microorganisms can decrease the cultivability of *L. pneumophila* but not the viability which indicates that the presence of autochthonous microorganisms can lead to misleading results when the safety of water is assessed by cultivable methods alone.

EPS and the microorganisms within are responsible for the occurrence of bad taste and odor, as well as depletion of dissolved oxygen, production of hydrogen sulfide, corrosion of distribution systems, and cloudy water while enabling the growth of pathogens.

Bacteria mostly associated with the development of EPS in water distribution systems are of the *phylum proteobacteria*, which are all gram-negative (lipopolysaccharide cell wall). From mature distribution systems, Biofilm samples consisted mainly of *Alphaproteobacteria* (26% of all phylotypes), *Gammaproteobacteria* (11%), candidate division *TM6* (11%), *Chlamydiales* (9%), and *Betaproteobacteria* (9%). The bulk water community consisted primarily of *Bacteroidetes* (25%), *Betaproteobacteria* (20%), *Actinobacteria* (16%), and *alphaproteobacteria* (11%). All biofilm communities showed higher relative abundances of single phylotypes and a reduced richness compared to bulk water. Only biofilm communities sampled at nearby sampling points showed similar communities irrespective of support materials. In bulk water studies, biofilms resulted in community compositions that were similar to each other. It is hypothesized that a higher fraction of active bacterial phylotypes and a better protection from oxidative stress in drinking water biofilms are responsible for this higher similarity.

The phylum *Alphaproteobacteria* are typically aerobes capable of growing at very low nutrient levels, have unusual methods of metabolism and may be rods, curved rods, spirals, *coccobacilli*, are pleomorphic in shape. Many species have extensions called “prosthecae”, which are used for attachment and can increase surface area for nutrient absorption. This genera includes nitrogen fixers, nitrifying bacteria, purple phototrophs. Pathogenic genera include *Rickettsia, Brucella* and *Ehrlichia*.

Alpha	*Acetobacter*	*Agrobacterium*	*Alcaligenes Bucella*	*Azospirillium*
	Beijerinka Ehrlichia	*Bradyrhizobium*	*Hyphomicrobium*	*Caulobacter*
	Nitrobcter	*Gluconobacter*	*Rhodosirillum*	*Methylocystis*
	Rhotobacter	*Paracocus*	*Rhodophia*	*Rhodopseudomonas*
	Rikettsia	*Rhodobacterium*	*Zymomonas*	*Rhizobium*
		Sphingomonas		

Phylum *Betaproteobacteria* are diverse and thrive on low levels of nutrients. They differ from *Alphproteobacteria* in their rRNA sequences, though metabolically some genera do overlap, i.e. nitrifying and nitrogen fixers. Noted pathogenic genera are *Neisseria*, *Bordetella* and *Burkholder.*

Beta	*Aquaspirillium*	*Bordetella*	*Burkholderia*	*Chromobacterium*
	Gallionella	*Leptothrix*	*Methylophylis*	*Neisseria Rhodocyclus*
	Nitrosomonas	*Oxalobacter*	*Ralstonia Spirillum*	*Thiobacillus*
	Rhodoferax Zoogloea	*Sphaerotilus*		

Phylum *Gammaproteobacteria* make up the largest and most diverse class of *proteobacteria*. Every shape, arrangement of cells, metabolic type and reproductive strategy is represented in this group. Included in the genera are purple sulfur bacteria, intracellular pathogens, methane oxidizers, facultative anaerobes, and Pseudomonads. The pathogens of note are *Legionella*, *Escherichia*, and *Vibrio*. Pathogens of this group are responsible for gastroenteritis, urinary tract infections, shigellosis, plague, pneumonia, cholera, and meningitis

Gamma	*Acetobacter*	*Acinetobacter*	*Azotobacter*	*Chromatium*
	Escherichia	*Ectothiorhodospira*	*Erwinia Legionella*	*Francisella*
	Halomonas	*Halorhodospira*	*Photobacterium*	*Leucothrix*
	Methylomonas	*Oceanospirillum*	*Nitrosococcus*	*Pseudomonas*
	Methylococcus	*Methylobacter*	*Vibrio*	*Thiobacillus*
	Thiomicrospira	*Salmonella*		*Xanthomonas*

*Delta proteobacteri*a is not a large group and is made up of sulfur reducing genera and genera that are parasitic in nature.

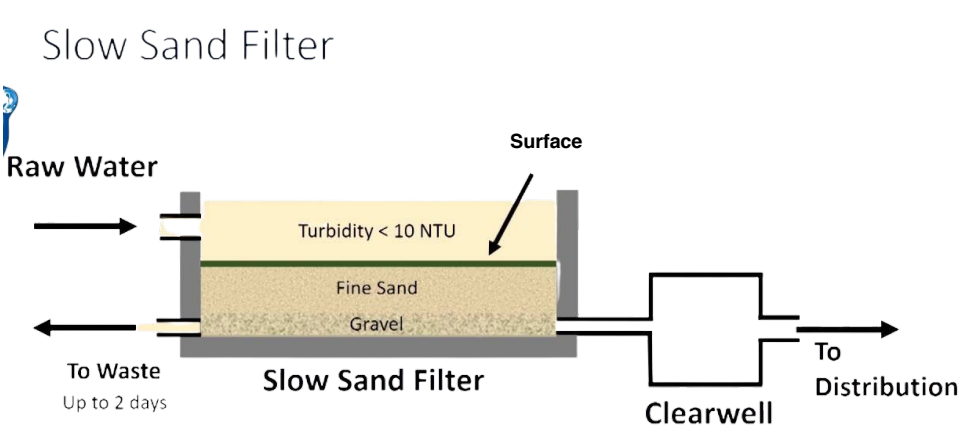
Delta	*Acinetobacter*	*Aeromonas*	*Bdellovibrio*	*Desulfuromonas*
	Francisella Mycoccous	*Geobacter*	*Halomonas*	*Moraxella*
		Pelobacter	*Syntrophobacter*	

Epsilonproteobacteri is a small group noted by the genera *Campylobacter*, a pathogen causing blood poisoning and gastroenteritis, and *Heliobacter*, which is a causative of gastric ulcers.

Epsilon	*Campylobacter*	*Heliobacter*	*Thiomicrospira*	*Thiovulum*
	Wolinella			

Sand Filters

EPS develops in sand filters rapidly. Within 2 days, CFU (colony forming units) densities reaching 1011 per gram along with abiotic and biotic particulates accumulated in the interstitial spaces. Bacteria (up to 90%) dominated the biofilm microbial community, with *Alphaproteobacteria* being the most common class.



As water percolates through the sand, abiotic and biotic particulates accumulate in the interstitial spaces. As biomass builds, a biofilm eventually forms on the surface of sand grains. The established biofilm can play a critical role in the transformation or degradation of a variety of harmful elements and compounds that occur in drinking water.

The interstitial microbial biomass decreases with depth, corresponding to a decrease of organic or inorganic compound concentrations as these compounds may act as a nutrient source for many microbial taxa.

Few published studies have focused on the role of microbes in drinking water treatment using sand filters, probably due to frequent backwashes and low substrate levels that are considered antagonistic to substantial colonization. However, microbial activity on the sand surface may be quite significant as biofilm develops under moderate temperature conditions.

Filamentous fungi associated with sand filters include *Alternaria alternata*, *Aspergillus niger*, *Cladosporium sp., Epiccocum nigrum, Fusarium sp*. and many *Penicillium sp*. are prevalent. *Penicillium brevicompactum* was rather frequently detected and *P. expansum* considerably less so.

Members of the *M. avium* complex and other mycobacteria have been recovered from natural waters and drinking water systems throughout the United States. The reported numbers of *M. avium* complex CFU per liter of sample have ranged from 0.8 to 100,000. *M. avium* complex organisms can grow in water and are highly resistant to ozone- and chlorine-based disinfectants. Further, *M. avium* numbers are higher in hot water systems than in the source waters, and a single *M. avium* clone was shown to persist for as long as 41 months in a single water distribution system. It is recognized that slow sand filtration is more efficient for removing mycobacteria than rapid sand filtration with the consequence of mycobacteria being added to the EPS within the filter. In addition, mycobacteria can colonize and grow on granular activated carbon and are able to enter distribution systems.

UV: Ultra-Violet Radiation

The action of UV radiation on microorganisms is to deactivate nucleic acids/proteins. Active sites on a protein are exposed due to complex conformations of the polymer. Deactivation is the unraveling of this conformation which then deactivates the functions of the protein, followed by cell death.

What determines the effectiveness of deactivation is UV wave-length, contact time and species of microbe. Some bacterial proteins can be re-activated downstream and protozoa require much higher doses and contact times than that of viruses and bacteria. As a result, U_V is often considered in concert with other chemical disinfectants.

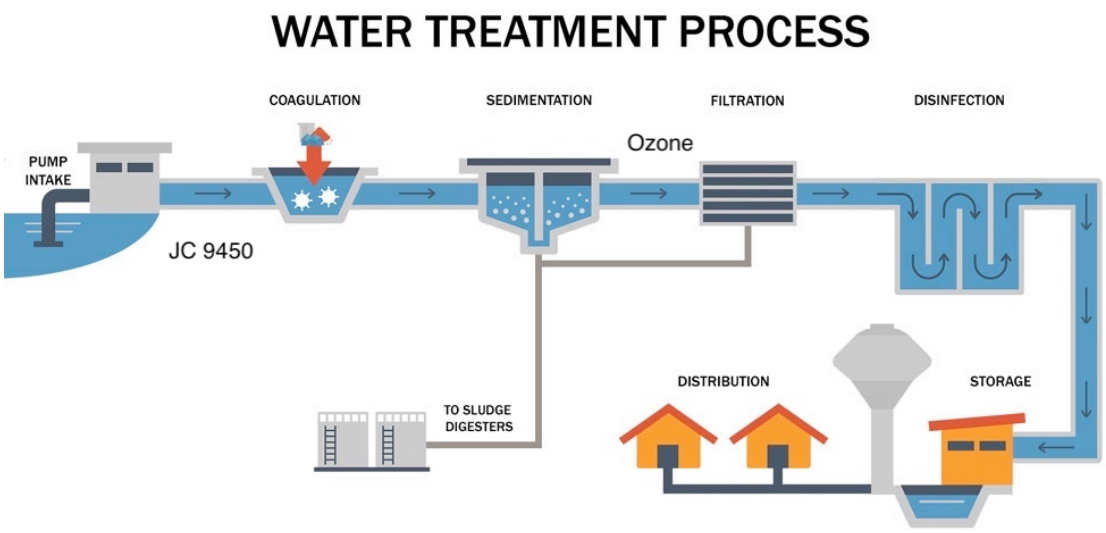
Anything that prevents U_V from making contact with microorganism will decrease the disinfection efficiency. Factors that are known to interfere with U_V efficiency are:

- Dissolved or suspended matter
- Iron, sulfites, nitrites and phenols all absorb U_V radiation
- Water has an absorbance coefficient which defines proximity of effectiveness
- Clumps of bacteria protected by EPS will not be affected by U_V
- Turbulence
- Upstream organics that collect on the U_V bulbs that encourage biofilm growth

Though U_V does not produce DBPs (Disinfection By-Products), the deactivated proteins become an AOC source feeding downstream biofilm microbial communities.

Water Distribution System

Once *proteobacteria* establishes EPS within the distribution system, the depletion of dissolved oxygen and the effects of disinfection, enables the establishment of gram-positive microbes, e.g*. Bacillus spp, Micrococcus spp, Nocardia spp, Rhodococccus spp, Staphylococcus spp,* to inhabit the EPS.



Prechlorination with a free chlorine residual results not only in reduced plate count values but also in a dramatic shift in the composition of the bacterial population to predominately gram-positive bacteria. Chlorination of biologically treated water produces the same shifts toward gram-positive bacteria. Removal of AOC by the biologically active filters slowed the rate of biofilm accumulation, but biofilm levels were similar to those found in conventionally treated water within several weeks. Iron pipes stimulated the rate of biofilm development, and bacterial levels on disinfected iron pipes exceeded those for chlorinated PVC pipes. Iron pipe surface dramatically influenced the composition, activity, and disinfection resistance of biofilm bacteria.

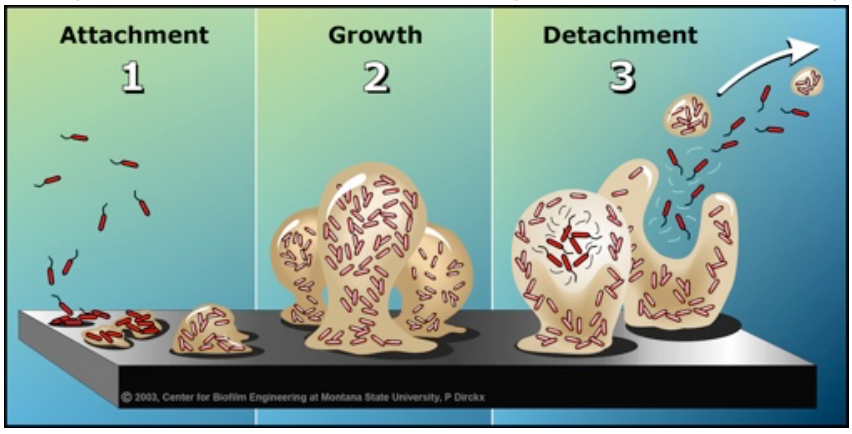
Analysis of the bacterial populations based on the Gram reaction showed that pre-chlorination resulted in a rapid shift from predominately gram-negative bacteria (97%) in the raw water to mostly gram positive-organisms (98%) in the chlorinated water. This predominately gram-positive population remained stable throughout the remainder of the treatment process.

Pre-ozonation, however, does not dramatically alter the composition of culturable raw water bacteria. Nearly 80% of the bacteria isolated from water treated with ozone at 3.2 mgs/ min/liter were gram negative, and the population of bacteria in the effluent of the biologically active GAC filter remained predominately (86%) gram negative. Exposure of biologically treated water to free chlorine resulted in the same shift to predominately gram-positive organisms as seen with conventional treatment.

Grouping of bacterial genera showed a diverse population of gram-negative bacteria in the raw-water sample. *Acinetobacter* spp., *Pseudomonas* spp., and *Klebsiella* spp. Re-dominated among the 20 genera identified in the raw water. Ozonation can reduced the microbial diversity to 13 genera, dominated by *Pseudomonas* spp., and *Rhodococcus* spp. Following biologically active GAC filtration, 19 genera were identified in the filter effluent, the majority of which (63%) matched isolates observed in the raw water. The predominant genera were *Pseudomonas* spp. and *Sphingomonas* spp. These organisms are widely distributed in the environment and can grow on a variety of carbon substrates. Chlorination of biologically treated water resulted in a shift to *Nocardia* spp. following distribution through the pipe system. Identification of isolates in the conventional treatment chain revealed 72% fewer genera than in the biologically treated

water, with *Nocardia* spp. dominating in the free- chlorinated water.

Rate of biofilm development. Despite the application of continuous disinfection, biofilms rapidly developed within a distribution system. For conventionally treated water, biofilm levels on cPVC pipe surfaces averaged 10^4 CFU/cm^2 within the first week. Biofilms attached to cPVC pipe surfaces in the biologically treated system developed at a lower rate, requiring 3 to 4 weeks to reach the same levels as in the conventionally treated system. Biofilm levels stabilized in both systems following the initial 3- to 4-week growth period. Biofilms rapidly form even in the presence of a 1- to 2-mg/liter free chlorine residual. Corrosion products have been previously shown to provide increased protection from free chlorine disinfection.



The hydrophobic-hydrophilic nature of the surfaces is known to affect the attachment of aquatic bacterial species to surfaces. Biofilm may be encouraged to develop on the surface of a plumbing material if that material is able to supply nutrient for bacterial growth, as is the case for latex. Plastic surfaces are known to leach metal ions at a sufficiently low level to prevent a toxic effect but could possibly contribute cations essential for enzyme function.Bacterial cells directly in contact with the materials are more likely to take up the ions. The plasticizers and other components of the pipe material may also be directly utilizable by some of the community of microorganisms in the biofilm and so contribute to the consortium as a whole.
The plastic surfaces were also capable of supplying some additional nutrient to the bacterial flora, but this was not the major cause of enhanced colonization of the plastic surfaces as compared to copper. The plastic materials has crevices and hollows on the surface as a result of their manufacture. These hollows were rapidly colonized, and areas of dense biofilm which eventually extended outwards were formed there. The initial colonization of these areas could be due to the protection from shear forces they gave to the colonizing bacteria.

The EPS serves as a focal point where bacterial and protozoal populations interact. The pioneering bacterial population will modify the surface conditions to enable bacterial succession to take place. The resultant EPS may aid colonization by *L. pneumophila* by supporting bacterial floras that provide essential nutrients by removing high inhibitory concentrations of oxygen by respiration or by encouraging protozoal populations which can act as hosts for the pathogen. However, the biofilm (or regions of it) may be inhibitory to *L.pneumophila* if bacterial flora produce extracellular products that inhibit growth directly or encourage a protozoal population that uses *L. pneumophila* as a preferential food source.

This process of modification of the biofilm flora would be continuing and dynamic until a stable climax community was achieved. The material used in the plumbing system wouldaffect this community by influencing the primary colonizing species and subsequent populations.

RO Membranes

The sequence observed in the colonization of new RO membrane and spacer surfaces is similar to biofilm formation on solid surfaces. The process consists of the following events:

(i) the transport of biological material to the surfaces, (ii) the attachment of primary colonizers, (iii) the initiation of early biofilm structures, and (iv) a spatiotemporal development into a multispecies slime layer with a complex three-dimensional architecture.Observed are two additional aspects: cells that mainly adhered in clumps and grew out as such and cells that mainly adhered as single cells and colonized the surface almost as a monolayer.

Relative abundances of the different species in the mature biofilm were different from those in the feed water, indicating that the biofilm was actively formed on the RO membrane sheets and was not the result of a concentration of bacteria present in the feed water.

The members of the *Alphaproteobacteria* subdivision in the biofilm presumably also originated from the mature biofilms of the upstream compartments of the plant. The genus *Sphingomonas* represented a major fraction (25%) of the sessile communities in the cartridge filter and ultrafiltration storage tank but was less dominant (7%) in the planktoniccommunity of the RO plant feed water, yet makes up 60% of the population

In contrast to the other pioneers, the majority of the *Alphaproteobacteria* colonizers, consisting of various *Sphingomonas* spp., were present as dispersed cells in the feed water of the RO system. Planktonic *Sphingomonas* cells have been reported to indicate depletion of suitable carbon sources and/or oxygen in the environment, i.e., oligotrophic conditions. Through the change from biofilm mode to planktonic mode, these bacteria are able to colonize new suitable environments. Traces of a broad range of naturally occurring organic compounds are supposed to be sufficient for growth, since sphingomonads are metabolically versatile organisms and have high-affinity uptake systems under nutrient-limiting conditions. It is postulated that after finding a suitable microenvironment, the *Sphingomonas*-like bacteria irreversibly attach by producing exopolysaccharides around their cells. This behavior leads to a relatively fast spreading of the cells over the membrane and spacer surfaces and make them the real colonizers of the membrane area. The wide spreading of the *Sphingomonas* EPS matrix over the membrane surface could well be due tothe shear stress caused by the fluid flow. Surface spreading also leads to enhanced substrate availability per cell compared to the availability of substrate to a dense packing and is advantageous in oligotrophic systems. The observed rapid spreading of the sphingomonads, concomitantly producing a layer of EPS on the surface, makes them a prime target for potential biofouling control approaches. They might not be the dominant organism in the fouling layer, but their almost unicellular layer and high level of EPS production likely gives them a more substantial contribution to membrane biofouling than aggregate-forming bacteria.

Reaction to disinfectants

The resistant mechanism of bacteria in biofilms can be attributed to several factors. Nutrient and oxygen depletion within the biofilm cause some bacteria to enter a nongrowing (ie, stationary) state, in which they are less susceptible to growth-dependent antimicrobial killing.

In addition, the presence of biofilm architectures serves as physical barriers to antimicrobial agents by blocking them from access to the bacteria in the deeper zone of the biofilm. However, the ability of EPS to act as a physical barrier depends on the type of disinfectant used, the concentration employed, and the binding of the matrix to that specific antibacterial agent. Strongly charged or chemically reactive agents fail to reach the bacteria in the biofilm due to the negatively charged EPS of biofilms which acts as an ion-exchange resin and removes these molecules from the environment. However, some small molecules such as water and oxygen have been found to be able to travel freely throughout the biofilm by using channels with varying diameters.

Following biofilm formation, sessile communities, subjected to 6-minute disinfection treatments with (i) benzalkonium chloride (50 ppm), (ii) sodium hypochlorite (10 ppm), (iii)peracetic acid (10 ppm), and (iv) a mixture of hydrogen peroxide (5 ppm) and peracetic acid (5 ppm) survive. *Listeria monocytogenes* and *Salmonella enterica* reached similar biofilm counts (ca. 10^5 CFU cm^2) and that, in general, interspecies interactions did not have any significant effect either on the biofilm-forming ability or on the antimicrobial resistance of each individual species. Additionally, the simultaneous existence inside the biofilm structure of *S. enterica* cells seemed to influence the occurrence and resistance pattern of *L.monocytogenes* strains. *Legionella pneumophila* is incapable of growth in sterile water because it requires nutrients to be supplied by other microorganisms, including bacteria (*predominantly Pseudomonas spp and Klebsiella spp*), amoebae, and cyanobacteria. The persistence of the pathogen in treated water systems has been attributed to the survival of the organism within.

H. pylori is more resistant than *E. coli* to chlorine and ozone at concentrations normally found within distribution systems. Thus, *H. pylori* cells entering a distribution system from outside sources or derived from biofilms within the system may be able to persist undetected in systems using either of these disinfectants.

The presence and number of *E. coli* do not correlate with any of physical and/or chemical characteristic of the drinking water (e.g., temperature, chlorine, or biodegradable organic matter concentration). *E. coli* is present in the biofilms of drinking water networks, some of the cells are metabolically active but are often not detected due to limitations of traditionally used culture-based methods, indicating that biofilm should be considered as a reservoir that must be investigated further in order to evaluate the risk for human health.

Cyanobacteria (blue-green algae) produce toxins that may present a hazard for drinking water safety. These toxins (microcystins, nodularins, saxitoxins, anatoxin-a, anatoxin-a(s), cylindrospermopsin) are structurally diverse and their effects range from liver damage, including liver cancer, to neurotoxicity. Ozone concentrations of at least 1.5 mg/L were required to provide enough oxidation potential to destroy the toxin presented by the cyanobacterium *Microcystis aeruginosa.* High raw water TOC will reduce the efficiency of free toxin oxidation and destruction. In addition, ozonation of raw waters containing high cyanobacteria cell densities will result in cell lysis and liberation of intracellular toxins. Thus,regular and simultaneous monitoring of TOC/dissolved organic carbon and cyanobacterial cell densities, in conjunction with online residual O_3 concentration determination and efficient filtration steps, can ensure the provision of safe drinking water from surface waters contaminated with toxic cyanobacterial blooms. However, these treatments may not be sufficient during bloom situations or when a high organic load is present, and toxin levels should therefore be monitored during the water treatment process.

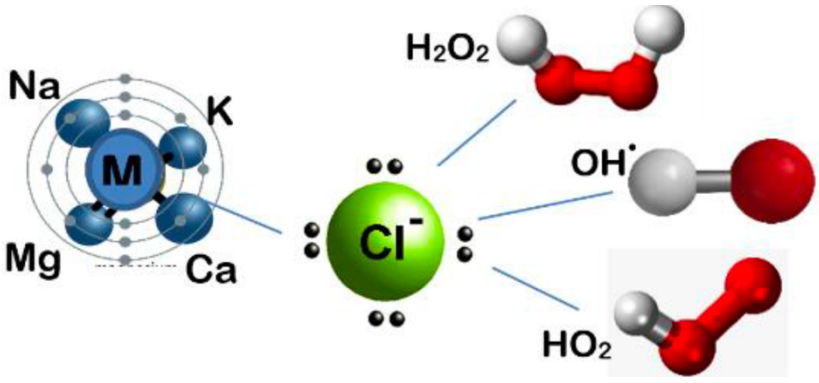
ROS: Reactive Oxygen Species

Bacteria are known to have protective mechanisms to ‘inactivate’ hydrogen peroxide through enzymes, (ie. catalase and peroxidase), which convert H_2O_2 into water and oxygen:

$$4\ \mathbf{H_2O_2 \rightarrow 2\ H_2O + O_2 \cdot 2\ H_2O_2 \rightarrow 2\ H_2O + O_2 + 2\ H_2O_2.}$$

These two active species, OH and H_2O_2, alone do not play a major role in the process of bacteria inactivation.

Charged species were shown to have a great influence on efficiency of bacteria inactivation, and several hypotheses have been proposed. First, it should be mentioned that energetic ions may be responsible for membrane etching. Another possible effect of ions is traditionally associated with superoxide ($^1O_2$), which in the presence of superoxide dismutase (SOD) is readily converted into hydrogen peroxide causing oxidative damage to the cell. Positive ions also may be converted into OH and hydrogen peroxide directly on the bacterial membrane. The third hypothesis is related to mechanical stress caused by electrostatic charging of bacteria. It was considered that bacteria exposed to ROS may be ruptured due to mechanical stress. Although in this model only spherical Gram-negative bacteria were considered, the proposed electrostatic rupture mechanism may be applied to



non-spherical microorganisms, which may be killed even easier. Another hypothesis is related to neutralization of bacterial surface charge, which leads to cytoplasm leakage and cell death.

These two last physical mechanisms of electrostatic inactivation of microorganisms may take place under conditions of an electrically isolated surface, where there is no leakage of transferred charge but accumulation of these charges. When bacteria were placed on the surface of conductive agar that was grounded, and therefore no charge accumulation may be expected. The results of this study clearly showed that inactivation of bacteria cannot be explained by the effect of only neutral active species or charged particles acting independently. Moreover, the presence of oxygen and water molecules with ROS is absolutely necessary. Therefore, a mechanism of peroxidation of bacterial membrane catalyzed by charges is proposed where both positive and negative ions have relatively thesame effect:

- the effect of charged species is *chemical* and not related to such physical phenomena as sheer stress, ion bombardment damage or thermal effects;
- ions *catalyze* peroxidation processes of a bacterial membrane composed of polysaccharides:
- the presence of *oxygen and water is necessary* and reactive oxygen species (ROS) play a crucial intermediate role.

Chain ion-radical mechanisms are well-known chemistry of hydrocarbons, where reactions of oxidation, peroxidation, hydrolysis, etc of organic molecules are catalyzed (and sometimes initiated by cation or anion molecules (ions). It is well known that these reactions can create long chains for both positive and negative ions and these chains may be much longer than the OH oxidation of organic molecules. Biophysical studies show that the greater catalytic effect of charged species is related to the various hydrogen atom transfer reactions, and could be due to both transition-state and product stabilization by increased solution ionic strength. At the same time, radicals derived from O_2 are believed tobe important in the initiation of lipid peroxidation-associated oxidative damage in biological systems. The role of water may be related to charged water clusters which carry both charge and radicals (e.g. OH and H_2O_2), resulting in simultaneous delivery of all active components to bacteria. Another possibility is related to production of highly reactive HO_2 ions, which are effectively transported to the target inside protective water clusters.

The role of charge in the process of bacteria inactivation is probably related to the activeradical transport to the treated surface. The ions (mainly OH^- $[H_2O]n$ and $H_3O^+[H_2O]n$)drift time to the surface is about 100– 200 μs. In contrast, convective transport with a typical speed of about 1 cm s^{-1} for the same distance of 4 cm requires about 4 s. Therefore, presence of charged particles provides about 4x orders of magnitude fastertransport of active radicals to the treated surface.

There are two possible mechanisms involved in the ROS mediated biofilm inactivation observed. The first involves the diffusion and penetration of ROS into the biofilms which caused the erosion of bacterial cells and resulted in severe damages to the cell membrane or to the DNA. The second mechanism involves the etching effect associated with ROS reactivity in contact with organics. In this mechanism, the ROS causes the chemical break down of the EPS. Once the architecture of biofilm is damaged, the EPS becomes unable to hold the content and bacteria (both dead and live) are released into the bulk solution. As the damage becomes more severe, detachment of biofilms from the solid substratum surface takes place. The results from kinetic studies reveal that the inactivation of biofilm byROS was through a more complex process which involved the two mechanisms.

CAUSES OF IRREVERSIBLE OXYGEN TOXICITY

Highly reactive and destructive by-products of oxygen reduction are invariably formed whenever oxygen is consumed by living cells and has the opportunity to react with manyreduced cellular constituents such as iron-sulfur proteins, thiols, tetrahydropteridines, flavoproteins, i.e., most organic compounds with double and conjugated double bonds.
Oxygen by-products are: hydrogen peroxide (H_2O_2), superoxide anion (O^-_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$).

$$O_2 + e^- \Rightarrow O_2^-$$

$$O_2^- + e^- + 2H^+ \Rightarrow H_2O_2$$

$$H_2O_2 + e^- + H^+ \Rightarrow H_2O + H^{\cdot}$$

$$OH + {}^{\cdot}e^- + H^+ \Rightarrow 2H_2O$$

Both peroxide (H_2O_2) and superoxide (O^-_2 are not of themselves dramatically cytotoxic, but,are particularly dangerous products of oxygen consumption because they can generate the more devastating hydroxyl radical (OH^-) via a biologically catalyzed sequence. The intracellular reduction of O_2 to $2H_2O$ requires addition of four electrons. This reduction usually occurs by single electron steps and the first product formed in the reduction of O_2 is*superoxide* anion, O^-_2.

Superoxide

Superoxide (O^-_2) is transiently produced by a one-electron transfer to oxygen by flavins, flavoproteins, quinones, thiols and iron-sulfur proteins. If the superoxide anion is not intercepted and neutralized, it will cause oxidative destruction to biochemical components within the cell. This anion has the longest life of the oxygen by-products and may pass from one cell to another. However, in chemical terms, O^-_2 is a poorly reactive radical in aqueous solution. The protonated form of O_2^-, hydroperoxyl radical, HO_2^-, is somewhat more reactive than O_2^-. For example, HO_2^- can initiate peroxidation of fatty acids. The equilibrium of HO_2^- with O_2^- is pH controlled and since the pH close to a membrane surface may be more acidic than the pH in bulk solution, HO_2^- formation will be favored. At a pH of 3.8, the ratio of O_2^- to HO_2^- is 1:10. At a pH of 5.8, the ratio shifts to 10:1, and at pH 6.8, the ratio leaps to 100:1. The pH beneath activated macrophages adhering to a surface has been reported to be 5 or less. Thus, a considerable amount of any generated superoxide will exist as HO^-. Because a cell membrane rejects charged molecules, $HO^{-\cdot}$ should be able to cross membranes as easily as H_2O_2. After penetration into the hydrophobic membrane interior, conditions favor O^- which is highly reactive in organic media and will attack carbonyl groupsof ester bonds that link fatty acids to the glycerol "backbone" of membrane phospholipids and are constituents of essential enzymes partly responsible for the biosynthesis of branch- chain amino acids

Hydroxyl

The hydroxyl radical, $OH^{\cdot}$, reacts with extreme high-rate constants with almost every type of molecule found in living cells, i.e., sugars, amino acids, phospholipids, nucleotides and organic acids. With saturated complexes, OH. will abstract a hydrogen to form water, leaving behind an unpaired electron on the carbon atom. This new carbon radical will undergo one or more additional reactions, e.g., alcohols will form a hydroxymethyl radical

capable of a wide range of reactions dependent on what might be available and sugars of DNA produce a huge array of different products, some of which become mutagenic. With more complex structures, i.e., aromatics and compounds with conjugated double bonds, $OH^{\cdot}$ will proceed by addition to the ring or double bond which drastically alters the physical properties from what is necessary for biosynthetic processes. Thus, hydroxyl radicals severely damage the bases and sugars of DNA and also induce strand breakage. If damage is repairable, mutations may result and if the damage is beyond repair, the cell will die.

The hydroxyl radical is a product from the endogenous reduction of peroxide by one of two methods.

1. $H_2O_2 + e^- + H^+ \Rightarrow H_2O + OH^{\cdot}$

2a. M^{3+} **-complex +** $O_2^{-} \Rightarrow M^{2+}$ **-complex +** O_2

2b. M^{2+} **-complex +** $H_2O_2 \Rightarrow OH^{\cdot} + OH^{-} + M^{3+}$ **-complex**

Where "M" is a metal, this reaction has become known as the *Haber-Weiss* reaction. Relating to bacteria, those species that produce superoxide dismutase (SOD), utilize metal chelates of iron and manganese in the process of building this enzyme. If concentrations of superoxide and peroxide are substantial enough to come in contact with a metal chelate, anhydroxyl radical can then be catalyzed. Especially among obligate anaerobes where the fermentative cycle requires substantial NADH, NADPH and thiol compounds, any interaction with metal ions and peroxide with these biosynthesis complexes will increase OH. formation.The observation of DNA damage induced by peroxide is hence mediated by some metal catalyzed Haber-Weiss reaction within the cell, i.e., H_2O_2 penetrates the plasma membrane and interacts with O_2^{-} generated intracellularly to form OH. , using metal ions bound to a weak metal chelate, metal containing enzyme or possibly to DNA.

Singlet Oxygen

Singlet oxygen is an excited form of molecular oxygen, annotated $^1O_2$, and is extremely reactive, via addition reaction with compounds containing carbon-carbon double bonds and conjugated double bonds, which are structural attributes of all biologically important substrates. A physical property which should be noted is that the decay rate of singlet oxygen to its ground state, O_2, competes with reactions of singlet oxygen with oxidizable substrates. In aqueous solutions, the decay rate is measured in micro-seconds, and in aprotic solvents (not containing hydrogen), the decay rate can be extended to 30 to 40 seconds.

In biologic systems $^1O_2$ is normally a by-product of specialized activity of cells associatedwith the immune system. Specialized enzyme reactions associated with saliva and phagocytosis produce singlet oxygen as part of the defense mechanism against invading microbes. Exogenous to a cell, sources of $^1O_2$ are usually the result of decomposition reactions whereby a free superoxide anion will attempt to react with anything it comes in contact with.

The cell wall of *gram-negative* bacteria has four layers. The outer coat is composed of *lipopolysaccharide* (LPS). LPS offers some protection from the toxic effects of exogenous agents. This capacity enables these bacteria to survive in hostile environments, i.e., gastrointestinal tract. LPS presents a physical/chemical barrier through which exogenous

$^1O_2$ must pass to interact with vital targets. Primarily, LPS repels $^1O_2$, but some does penetrate this layer and becomes trapped among the unsaturated fatty acids and protein components wherein peroxidation will occur. All things not being equal, some strains fail to produce a significant LPS layer which increases their sensitivity to exogenous $^1O_2$. Most *gram-positive* bacteria have a bi-layer membrane with an outer coat of *peptidoglycan* (PG),which with greater frequency, allows substantially more $^1O_2$ to pass through than LPS. For both types of bacteria, when $^1O_2$ traverses the membrane layers any number of enzyme/protein deactivation reactions can occur and when enough enter within a bacterium, i.e., more than can be countered, death is certain.

The toxicity of $^1O_2$ is hence dependent on the number of molecules attacking a bacterium. Calculations have concluded that to achieve a 99% kill, 1.3×10^{-5} mol of singlet oxygen should reach a bacterium in 20 minutes. On average, gram-negative bacterium required 5×10^{10} molecules of $^1O_2$ per cell and gram-positive bacterium required 6×10^9 molecules $^1O_2$ per cell. This finding suggests a low probability of vital target-singlet oxygen interaction, so most collisions do not result in cell death. However, the probability that the inactivating collision will occur never changes. A single reaction of $^1O_2$ could conceivably have devastating global effects such as initiating lipid peroxidation and subsequent radical- mediated reactions. Single hit kinetics in this case are not likely due to lethal DNA damage, as $^1O_2$ does not readily react with bacterial DNA.

ROS Discussion

Both hydroxyl radical and singlet oxygen are indiscriminate reactive reagents and their cytology is enhanced when they are generated in an *aprotic* solvent, e.g., within lipid membranes wherein their half-lives would be extended and molecular oxygen would be more soluble. The bactericidal effects of these radicals are the result of breaks in DNA, lipidperoxidation, impairment of transport processes across membranes, destruction of key enzymes and co-factors of reductant and energy generating pathways and/or biosynthetic processes. The suggestion is that obligate anaerobes depend on metabolic processes madeup of excessively oxygen sensitive enzymes and co-factors and are either void of or lack sufficient means to neutralize oxygen radicals as they may be produced endogenously or introduced exogenously.

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When oxygen and its by-products overwhelm a bacterium, the following sequence of events takes place.

1. Oxidation of scavengers.
2. Peroxidation/disruption of membrane layers.
3. Oxidation of thiol groups.
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JC 9450: OXIDATION POTENTIAL

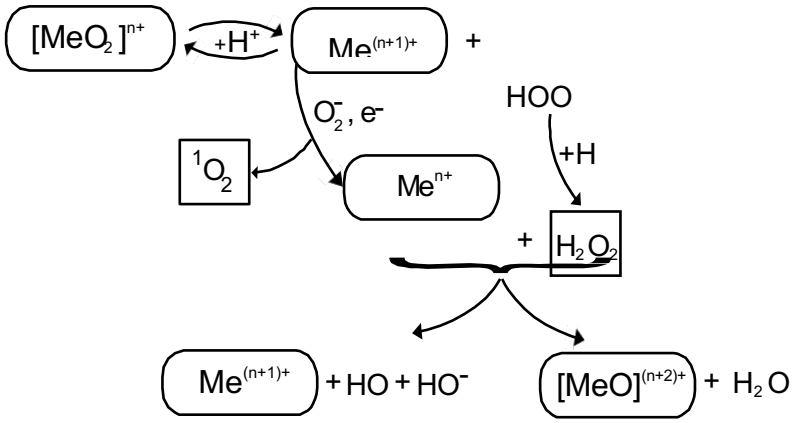
JC 9450 is a complex mineral-oxy-chloride solution that forms metal-oxy-complexes, which are diamagnetic molecules, that remove restrictions for the oxygen reactions with molecules in the singlet state. These complexes also decrease the ionic activity of the metalions. In general, there is a stabilization against reduction of metal ions (of low concentration) by these complex formations. Coordination with a donor group increases theoxidation potential and thereby increases the relative stability of the higher oxidation states(valences).

Metal-oxy-complexes cause an increase in the oxidation properties [potential] of the oxygen complex compared to the oxygen molecule alone. The change in the redox behavior of the metal ion, and its oxidation state, results from the interaction with oxygen, the type of ligand formed and its field geometry.

Iron and manganese will form oxy-complexes that will release superoxide, hydroxyl and nascent oxygen ions. These reactions cause a valence change in the metal, which then catalyses the reaction with superoxide to produce peroxide. Peroxide, in the presence of Fe/Mn ions, will decompose to hydroxyl radicals. The interconversions of these ions produces nascent oxygen.

$$Me^{[n+1]+} + O_2^- \; Me^{n+} + H_2O_2 \; HO^{\cdot} + HO^- + Me^{[n+1]+}$$

The stability of the oxy-complexes strongly depends on the pH and the matrixes formed between and with minerals and metals (Ca, K, Cl, Fe, Mn, Mg). In the presence of proton donors, they undergo decomposition to yield peroxide. Furthermore, it is generally accepted that peroxide yields hydroxyl radicals as a result of catalytic reactions in the presence of metal ions. By this mechanism, the oxy-complex of the metal would produce hydroxyl radicals on contact with organic material at the point where the metal is bound. This reaction will also place the metal in a higher valence state, which is also a highly oxidizing species.



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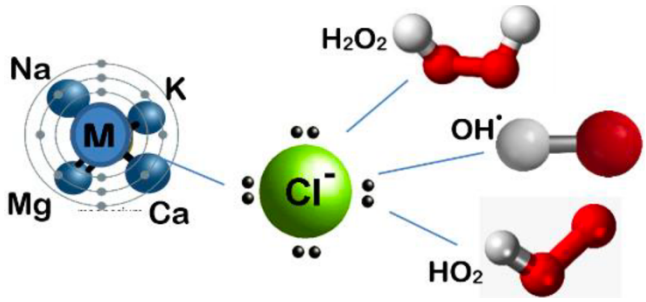
The formation of metal oxy-complexes results in the loss of "normal" chemical reactions of metal ions in solution. The fact that metal ions do not behave as expected is an indication that these ions are present in very low concentrations. What would normally precipitate a metal ion from a solution, will not work with metal-oxy-complexes because the metal, whilein an oxy-complex formation, is "deactivated" from its expected behavior. Metals are normally expected to precipitate as an oxide, but the formation of mineral-metal-oxy-ion, *shelled by water*, keeps the metal in solution, thus de-activating the metal from normal behavior. Under this condition, no precipitation occurs until the concentration of the metal ion rises to a value such that the solubility product of the insoluble salt is exceeded.

Di-positive ions, (Ca, Fe, Mg, Mn) will form monohydroxy complexes and at pH above 9, these oxy-complexes are *continuously converted* to oxy-complexes having two or morehydroxyl groups. They also form interconnected matrices of a transient nature.



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It is known that an ion has a negative contribution of entropy (randomness) which is due tothe restriction of degrees of freedom of water molecules in the vicinity of the ion and that the effect is greater the greater the charge of the ion. When a metal in a simple ionic state is oxidized, the entropy contribution decreases. Conversely, an increase in valence of a metal ion, when it exists in a negative anion (oxy-complex), results in a decrease in the charge of the ion and in a corresponding entropy increase.

The increased entropy (randomness of structure) means that the oxidation state of both the metal and oxygen ions are maintained by the resonance of complex and rapidly changing matrixes of these ion complexes.

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Water, by forming a shell around a metal-oxy-complex, enables the complex to *retain its energy* for extended times (residual ORP). This water shell, because of its hydrogen bondingwith organics, becomes a bridge that delivers the metal-oxy-complex to a weak spot on the organic compound. Depending on the properties of the organic reactant, the water shell willeither help the metal-oxy-complex regenerate (regain active oxygen species) or decompose(loose all active oxygen molecules).

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Discussion

Suffice it to say that the micro-environment of bacterium is complex, as well as their inter-relationships and coping mechanisms to cope with environmental and chemical stressors. For a disinfection program to be successful, it must address not only the bacterium, but alsothe survival mechanisms presented by microbial populations. It is the ability to deliver ROS that effectively and efficiently contends with these issues.

Because the nature of chlorine is to undergo addition reactions, that is, it likes to attach itself to organics, its ability to “oxidize” is measured in ppm, NOT by the oxidation energy itprovides. Chlorine, at a pH of 4-5, produces hypochlorous acid, which is the desired disinfectant that is active against all microorganisms. To use chlorine correctly a water stream must be prepared to receive a chlorine compound to achieve optimum effectiveness.

Factors affecting Chlorine Activity:

1. pH: MUST be adjusted to pH of 4-5,. By adjusting the pH, the bicarbonates become carbonates and cease to scavenge oxidants (bicarbonates are oxidant scavengers).

2. Temperature: chlorine reactivity is temperature sensitive. Cold water slows downand requires more chlorine than warmer water (well vs surface, summer vs winter).
3. Organics: Organics will consume chlorine to form *chloramines and THMs'*
4. Ammonia & Nitrates: these two compounds will slow the bacterial kill action of chlorine, increasing the contact time required for chlorine to work.

Reactivity of Chlorine Compounds

The reactivity of chlorine has been well studied and general guidelines for its use are asfollows:

1. Inorganics: Chlorine, used by itself, requires a concentration of *6 mg/L per 1.0 mg/L of inorganic at a pH of 8.5 –9.0*. If used in conjunction with permanganatethis ratio will drop to below 1.0 mg/L per 1.0 mg/L of inorganic but will require tight instrumentation controls.
2. Pathogens: At a pH of 4-5, chlorine is effective as a disinfectant when used at a ratio of 1-3 mg/L per 0.5 mg/L pathogen *and requires a residence time of 45 –60 minutes (12 time that for Ozone).*
3. Pathogens: A 500 mg/L chlorine concentration will normally be effective against vegetative bacteria. At this concentration, chlorine has limited effect against viruses and is poorly effective against bacterial spores and fungi (*adverse effectson body chemistry/metabolism).*
4. Pathogens: a 1000 mg/L chlorine concentration is effective against all bacteria, viruses and fungi yet requires 25-30 minute residence time (*adverse effects on body chemistry/metabolism).*
5. Organics: practical applications of chlorine, even at 1000 mg/L have little effecton oxidizing organic material other than generating chlorinated organics, hence chlorine is not recommended for this use.
6. Residual: A chlorine residual can be provided only after the demand for chlorineby inorganic, bacteria and organics has been met

Chlorine can be effective for oxidizing inorganics and bacteria when pH, concentration and residence time are allowed for, **but** the chlorinated organic by-products are inevitable and only some may be filtered out. For potable and waste water applications, the important consideration for utilizing chlorine compounds is the amount of chlorides that will end up being consumed and the impact of the chlorinated organics being consumed or dumped into the environment. If excess chlorides are produced, necessary steps should be taken to remove them as a last step in a water treatment program.

JC 9450: Disinfectant for the 21st Century

Advanced physical and chemical treatments such as disinfection, flocculation, chlorination, coagulation, sedimentation, filtration, refining, UV irradiation and ozonation, irradiation with UV, ozonation and chlorination, have all been unsuccessful at eliminating biofilm growth on strategic surfaces in a water/wastewater treatment facility because of the inability to distribute sufficient oxidation energy (ORP) to contend with bacterial EPS and the AOC that feeds microbial colonies.

Biofilm before JC 9450 addition

Chlorine dioxide can provide an effective ROS that will oxidize EPS and disinfect microbes, but direct and indirect costs are prohibitive. Likewise Ozone has proved to be effective, but being a highly reactive gas, it is only useful near the point of injection and cannot provide sufficient downstream ORP.

JC 9450 provides mineral/metal oxy-complexes that provide a variety of ROS in responseto the microenvironment on the surfaces of EPS, organics and microbes. Because **JC 9450** is a chelation of minerals with oxygen in liquid form, it has a readily measurable ORP. The **JC 9450** oxy-complexes work with the polymerization property of water to make shells around these oxy-complexes, to deliver and attenuate these ionic conformations such thatwhen it comes in contact with inorganics, microorganisms and organic matter, it readily gives off ROS that aggressively oxidizes all desired contaminants. The added benefit of **JC 9450** is that the metals used act as catalysts for ROS reactions, hence both aiding in the conservation of ORP and enhancing ORP. The reactivity of **JC 9450** is closely matched to ozone but without the problems associated with dissolving a gas in water and is able to provide residual ORP.

Reactivity of JC 9450

1. Inorganics: A concentration of less than 1.0mg/L per mg/L of inorganic
2. Pathogens: A concentration of 1.0 mg/L per 1,000 – 10,000 mg/L pathogen
3. Organics: A concentration of 1.0 – 8.0 mg/L per 1.0 mg/L organics
4. Residual: The byproducts of **JC 9450** are mineral oxides that are effective against bacterial recontamination hence providing a protective residue that kills bacteria.

One uniqueness of **JC 9450** is that it releases ROS imbedded within the molecular structureof organic material and the molecular make up of pathogens. Therefore, **JC 9450** perpetuate the release of highly active ROS.

JC 9450 capitalizes on the properties and principles of both “Haber-Weiss” and “Fenton” reactions. These reactions define the relationship between ROS and how minerals/metals mitigate ROS reactions. These reactions are descriptive of natural events in biologic systems anddemonstrate the effectiveness of ROS reactions and of **JC 9450’s** ROS in killing microbes, oxidizing organics, versus the poisoning a microbe with chlorine or increasing the toxicity of organics via the addition of chlorine.

By mimicking nature, by utilizing high energy ROS, **JC 9450** easily breaks down nature’s defenses to kill microbes. And by utilizing metabolically friendly minerals and metals, **JC 9450** residues readily break down and are consumed by nature.

Measuring ORP is the key to utilizing the potential of **JC 9450**. A primary advantage is thatusing ORP for water system monitoring provides the operator with a rapid and single-value assessment of the disinfection potential of water without having to test for and calculate organic loading. Versus conventional disinfectants, **JC 9450** can accomplish a desired ORP disinfection and oxidation of EPS, organics and inorganics with less product and because ofthe effectiveness of **JC 9450**, its residues and byproducts fall well below FDA's MCL (maximum contaminant level).
on the RO membrane.

take place under conditions of an electrically isolated surface, where there is no leakage of transferred charge but accumulation of these charges. When bacteria were placed on the surface of conductive agar that was grounded, and therefore no charge accumulation may be expected. The results of this study clearly showed that inactivation of bacteria cannot be explained by the effect of only neutral active species or charged particles acting independently. Moreover, the presence of oxygen and water molecules with ROS is absolutely necessary. Therefore, a mechanism of peroxidation of bacterial membrane catalyzed by charges is proposed where both positive and negative ions have relatively thesame effect:

- the effect of charged species is *chemical* and not related to such physical phenomena as sheer stress, ion bombardment damage or thermal effects;
- ions *catalyze* peroxidation processes of a bacterial membrane composed of polysaccharides:
- the presence of *oxygen and water is necessary* and reactive oxygen species (ROS) play a crucial intermediate role.

Chain ion-radical mechanisms are well-known chemistry of hydrocarbons, where reactions of oxidation, peroxidation, hydrolysis, etc of organic molecules are catalyzed (and sometimes initiated by cation or anion molecules (ions). It is well known that these reactions can create long chains for both positive and negative ions and these chains may be much longer than the OH oxidation of organic molecules. Biophysical studies show that the greater catalytic effect of charged species is related to the various hydrogen atom transfer reactions, and could be due to both transition-state and product stabilization by increased solution ionic strength. At the same time, radicals derived from O_2 are believed tobe important in the initiation of lipid peroxidation-associated oxidative damage in biological systems. The role of water may be related to charged water clusters which carry both charge and radicals (e.g. OH and H_2O_2), resulting in simultaneous delivery of all active components to bacteria. Another possibility is related to production of highly reactive HO_2 ions, which are effectively transported to the target inside protective water clusters.

The role of charge in the process of bacteria inactivation is probably related to the activeradical transport to the treated surface. The ions (mainly OH^- $[H_2O]n$ and $H_3O^+[H_2O]n$)drift time to the surface is about 100– 200 μs. In contrast, convective transport with a typical speed of about 1 cm s^{-1} for the same distance of 4 cm requires about 4 s. Therefore, presence of charged particles provides about 4x orders of magnitude fastertransport of active radicals to the treated surface.

There are two possible mechanisms involved in the ROS mediated biofilm inactivation observed. The first involves the diffusion and penetration of ROS into the biofilms which caused the erosion of bacterial cells and resulted in severe damages to the cell membrane or to the DNA. The second mechanism involves the etching effect associated with ROS reactivity in contact with organics. In this mechanism, the ROS causes the chemical break down of the EPS. Once the architecture of biofilm is damaged, the EPS becomes unable to hold the content and bacteria (both dead and live) are released into the bulk solution. As the damage becomes more severe, detachment of biofilms from the solid substratum surface takes place. The results from kinetic studies reveal that the inactivation of biofilm byROS was through a more complex process which involved the two mechanisms.

ROS Discussion

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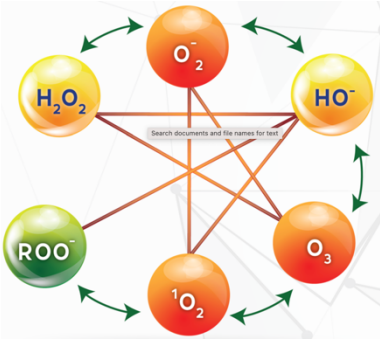

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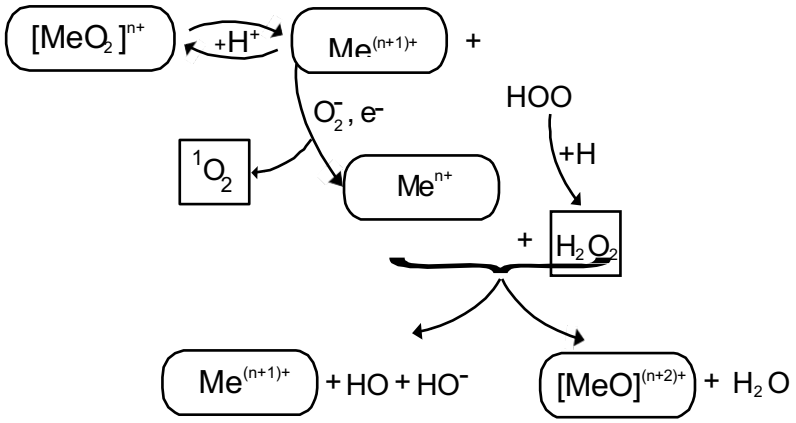

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The reactivity of chlorine has been well studied and general guidelines for its use are asfollows:

7. Inorganics: Chlorine, used by itself, requires a concentration of *6 mg/L per 1.0 mg/L of inorganic at a pH of 8.5 –9.0*. If used in conjunction with permanganatethis ratio will drop to below 1.0 mg/L per 1.0 mg/L of inorganic but will require tight instrumentation controls.
8. Pathogens: At a pH of 4-5, chlorine is effective as a disinfectant when used at a ratio of 1-3 mg/L per 0.5 mg/L pathogen *and requires a residence time of 45 –60 minutes (12 time that for Ozone).*
9. Pathogens: A 500 mg/L chlorine concentration will normally be effective against vegetative bacteria. At this concentration, chlorine has limited effect against viruses and is poorly effective against bacterial spores and fungi (*adverse effectson body chemistry/metabolism).*
10. Pathogens: a 1000 mg/L chlorine concentration is effective against all bacteria, viruses and fungi yet requires 25-30 minute residence time (*adverse effects on body chemistry/metabolism).*
11. Organics: practical applications of chlorine, even at 1000 mg/L have little effecton oxidizing organic material other than generating chlorinated organics, hence chlorine is not recommended for this use.
12. Residual: A chlorine residual can be provided only after the demand for chlorineby inorganic, bacteria and organics has been met

Chlorine can be effective for oxidizing inorganics and bacteria when pH, concentration and residence time are allowed for, **but** the chlorinated organic by-products are inevitable and only some may be filtered out. For potable and waste water applications, the important consideration for utilizing chlorine compounds is the amount of chlorides that will end up being consumed and the impact of the chlorinated organics being consumed or dumped into the environment. If excess chlorides are produced, necessary steps should be taken to remove them as a last step in a water treatment program.

JC 9450: Disinfectant for the 21st Century

Advanced physical and chemical treatments such as disinfection, flocculation, chlorination, coagulation, sedimentation, filtration, refining, UV irradiation and ozonation, irradiation with UV, ozonation and chlorination, have all been unsuccessful at eliminating biofilm growth on strategic surfaces in a water/wastewater treatment facility because of the inability to distribute sufficient oxidation energy (ORP) to contend with bacterial EPS and the AOC that feeds microbial colonies.

Chlorine dioxide can provide an effective ROS that will oxidize EPS and disinfect microbes, but direct and indirect costs are prohibitive. Likewise Ozone has proved to be effective, but being a highly reactive gas, it is only useful near the point of injection and cannot provide sufficient downstream ORP.

JC 9450 provides mineral/metal oxy-complexes that provide a variety of ROS in responseto the microenvironment on the surfaces of EPS, organics and microbes. Because **JC 9450**is a chelation of minerals with oxygen in liquid form, it has a readily measurable ORP. The **JC 9450** oxy-complexes work with the polymerization property of water to make shells around these oxy-complexes, to deliver and attenuate these ionic conformations such thatwhen it comes in contact with inorganics, microorganisms and organic matter, it readily gives off ROS that aggressively oxidizes all desired contaminants. The added benefit of **JC 9450** is that the metals used act as catalysts for ROS reactions, hence both aiding in the conservation of ORP and enhancing ORP. The reactivity of **JC 9450** is closely matched to ozone but without the problems associated with dissolving a gas in water and is able to provide residual ORP.

Reactivity of JC 9450

5. Inorganics: A concentration of less than 1.0mg/L per mg/L of inorganic
6. Pathogens: A concentration of 1.0 mg/L per 1,000 – 10,000 mg/L pathogen
7. Organics: A concentration of 1.0 – 8.0 mg/L per 1.0 mg/L organics
8. Residual: The byproducts of **JC 9450** are mineral oxides that are effective against bacterial recontamination hence providing a protective residue that kills bacteria.

One uniqueness of **JC 9450** is that it releases ROS imbedded within the molecular structureof organic material and the molecular make up of pathogens. Therefore, **JC 9450** perpetuate the release of highly active ROS.

JC 9450 capitalizes on the properties and principles of both “Haber-Weiss” and “Fenton” reactions. These reactions define the relationship between ROS and how minerals/metals mitigate ROS reactions. These reactions are descriptive of natural events in biologic systems anddemonstrate the effectiveness of ROS reactions and of **JC 9450’s** ROS in killing microbes, oxidizing organics, versus the poisoning a microbe with chlorine or increasing the toxicity of organics via the addition of chlorine.

By mimicking nature, by utilizing high energy ROS, **JC 9450** easily breaks down nature’s defenses to kill microbes. And by utilizing metabolically friendly minerals and metals, **JC 9450** residues readily break down and are consumed by nature.

Measuring ORP is the key to utilizing the potential of **JC 9450**. A primary advantage is thatusing ORP for water system monitoring provides the operator with a rapid and single-valueassessment of the disinfection potential of water without having to test for and calculate organic loading. Versus conventional disinfectants, **JC 9450** can accomplish a desired ORP disinfection and oxidation of EPS, organics and inorganics with less product and because ofthe effectiveness of **JC 9450**, its residues and byproducts fall well below FDA's MCL (maximum contaminant level).
on the RO membrane.

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